LDLR NEGATIVE PACKAGING CELL LINE FOR THE PRODUCTION OF VSV-G PSEUDOTYPED RETROVIRAL VECTOR PARTICLES OR VIRUS PARTICLES THEREOF

REFERENCE TO RELATED APPLICATIONS

[0001] This application is the U.S. National Stage of international application PCT/EP2019/072931, filed Aug. 28, 2019 (pending) and published on Mar. 5, 2020 as WO 2020/043765; which claims the priority benefit of EP application 18191784.0. The PCT application is hereby incorporated herein by reference in its entirety for all purposes.

Field of the invention

[0002] The present invention relates to the field of packaging cell lines for production of retroviral vector particles or virus like particles thereof, especially for packaging cell lines that do not express the Low-Density Lipoprotein Receptor (LDLR) on their surface for the production of VSV-G pseudotyped retroviral vector particles or vectorlike particles (VLP) thereof.

BACKGROUND OF THE INVENTION

[0003] In the field of T and stem cell gene therapy, retroviral vectors such as lentiviral vectors (LV) are considered a highly efficient tool to deliver therapeutic nucleic acid molecules to target cells and induce long-term expression. Usually, retroviral vectors contain heterologous envelope proteins from foreign virus species within the retroviral membrane. The process of exchanging viral vector envelope proteins is called "pseudotyping". The most commonly used envelope protein for pseudotyping is the G protein of the *Vesicular stomatitis* Virus (VSV-G). It transduces a broad range of target cells including therapeutic relevant cell types like stem cells and T cells. Binding of the trimeric VSV-G protein to its receptors induces multiple conformational changes from the pre-fusion to the post fusion state to catalyze the insertion of a hydrophobic fusion peptide to the target cell membrane and subsequent fusion of viral and target cell membrane. This process is pH dependent meaning that fusion of the viral and cellular membrane takes place predominantly in the endosome.

[0004] Before 2013, the attempts to identify the VSV-G receptor were not conclusive. For example, it has been proposed that the receptor is a phospholipid and not a protein. Then, Finkelshtein et al, Amirache et al and Nikolic et al have confirmed that LDLR is the main receptor of VSV-G. In addition, related family members of LDLR (i.e. LRP1, LRP1b, LRP2, LRP4, LRP5 and LRP6, VLDLR, LRP8) contribute to VSV mediated binding and infection as well. LDLR is a type 1 transmembrane glycoprotein and responsible for the regulation of cholesterol homeostasis in mammals. Loss-of-function mutations of the LDLR gene are associated with impaired delivery of cholesterol-rich LDL from the blood to the cells, which may result in familial hypercholesterolaemia in human. LDL is bound under neutral pH, followed by receptor-ligand internalization which finally leads to the release of the ligand in the endosome under acidic conditions. Afterwards the receptor is recycled back to the cell surface. Otahal et al has confirmed that the ligand-binding domain of LDLR plays a critical role for VSV-G interaction. Nikolic et al more specifically identified two distinct cysteine rich domains (CR2 and CR3) being responsible for binding to VSV-G as well. Related CR domains are also found in the VLDLR, LRP1, LRP1B, LRP2, LRP3, LRP4 (Nikolic et al). For enveloped viral particles like lentiviral vectors sufficient supply with lipids and the lipid composition of the packaging cell membrane are critical parameters of the infectivity and the yield of the viral particles (Guyader et al).

[0005] State-of-the-art protocols for VSV-G pseudotyped retroviral vectors are based on packaging cells like HEK 293 cells and its derivatives. These packaging cells are mainly cultivated in adherent cultivation vessels like roller bottles or cell factories but suspension cell cultures are of great interest to address limitations in scalability.

[0006] Stable packaging cells for VSV-G pseudotyped retroviral vectors continuously expressing some or all components are not available so far. This is attributed to the well-known toxicity of VSV-G (Rodrigues, Alves and Coroadinha); Thus, transient protocols have been established in the field that are based on transfecting packaging cells with 3-5 plasmids encoding all components (Merten et al). This approach enables a high level of flexibility by simply exchanging the required plasmids. However the reproducibility in yield from lot to lot is rather low due to variations in transfection efficiencies.

[0007] To avoid transfection procedures but to enable a high level of reproducibility, inducible systems have been established in the art to restrict expression of the toxic VSV-G protein to the harvesting period only. These systems require sophisticated transcriptional control expression cassettes and substances specifically inducing or shutting down the transcription of the gene of choice. Most systems make use of Tet-On/Tet-Off systems but alternative systems based on e.g. hormones are reported as well. These systems do not solve the problem of cytotoxicity but avoid the need of transient transfection and limit the burden of VSV-G toxicity to the harvesting period only. However, additional purification might be needed for therapeutic application to deplete the substance from the viral harvest. In addition, inducible or stable producing systems typically require large screening campaigns to identify packaging cell clones expressing all components at an optimal ratio to harvest high yields of retroviral vectors.

[0008] Thus, there is a need in the art for an improved or alternative packaging cell line for the production of VSV-G pseudotyped retroviral vector particles or virus like particles thereof and for the VSV-G pseudotyped retroviral vector particles or virus like particles thereof produced by said packaging cell line.

SUMMARY OF THE INVENTION

[0009] Surprisingly, the inventors found that a packaging cell line for the production of VSV-G pseudotyped retroviral vector particles or virus like particles thereof leads to higher yields of VSV-G pseudotyped retroviral vector particles or virus like particles thereof when said packaging cell line naturally or genetically engineered does not express the LDLR, i.e. the packaging cell line is negative for LDLR, as compared to a packaging cell line that expresses LDLR (an LDLR positive packaging cell line; e.g. HEK 293T WT). Said VSV-G pseudotyped retroviral vector particles or virus like particles may be VSV-G pseudotyped lentiviral vector particles or virus like particles and/or VSV-G pseudotyped gamma-retroviral vector particles or virus like particles.